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Article *in* Palynology · March 2022

DOI: 10.1080/01916122.2022.2047818

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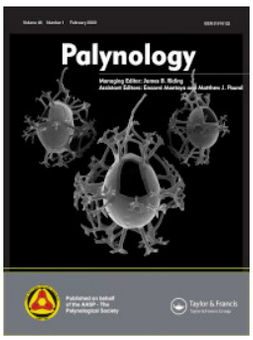

Palynology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tpal20

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To cite this article: Talita Kely Bellonzi & Eduardo Custódio Gasparino (2022): Pollen morphology of Napeantheae Wiehler (Gesneriaceae) from Brazil, Palynology, DOI: 10.1080/01916122.2022.2047818

To link to this article: https://doi.org/10.1080/01916122.2022.2047818

PALYNOLOGY
https://doi.org/10.1080/01916122.2022.2047818

Pollen morphology of Napeantheae Wiehler (Gesneriaceae) from Brazil

Talita Kely Bellonzi[a] and Eduardo Custódio Gasparino[b]

[a]Faculdade de Filosofia, Ciências e Letras, Programa de Pós-Graduação em Biologia Comparada, Universidade de São Paulo (USP), Ribeirão Preto, SP, Brazil; [b]Faculdade de Ciências Agrárias e Veterinárias, Departamento de Biologia Aplicada à Agropecuária, Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brazil

ABSTRACT

Napeantheae is a monogeneric tribe of Gesneriaceae (with *Napeanthus* Gardner), that presents species with different morphology from other Gesnerioideae, being herbs with rosulate leaves that grow in pits, banks or at the base of rocks in humid forests. The tribe's palynology is poorly known. Thus, the aim of this study was to investigate the pollen morphology of the Brazilian native species of *Napeanthus* (Napeantheae) in order to expand the morphological knowledge of the tribe and compare it with existing studies on Gesneriaceae. For the analysis, the pollen grains were removed from flower buds of exsiccatae deposited in herbaria; they were acetolyzed, measured, photographed using light microscopy (LM) and scanning electron microscopy (SEM), and described qualitatively. The analyzed species present differences in amb, polar area, shape, size of ectoapertures, type of endoapertures and exine thickness. The pollen grains are monads, isopolar, small, tricolporate, circulaperturate, ectoaperture rounded at the polar ends and without margo; the exine is semitectate, microreticulate-fossulate, sexine is thicker than nexine. An artificial pollen key is presented to help distinguish species. Quantitative pollen grain data confirm the qualitative differences observed, especially in relation to the type of endoaperture. The microreticulate-fossulate exine ornamentation observed in *Napeanthus* has not previously been recorded for Gesneriaceae, especially in Gesnerioideae.

KEYWORDS

Brazilian species; Gesnerioideae; palynology; palynotaxonomy; pollen grains

1. Introduction

Gesneriaceae Rich & Juss. ex DC. (Euasterids I, Lamiales) have 147–148 genera and approximately 3260 species (Wiehler 1983; Weber 2004), widely distributed, are well represented in the tropics, with few native species in temperate regions (Burtt and Wiehler 1995) and center of diversity in Colombia to Ecuador, followed by the Central American region and southeastern Brazil (Perret et al. 2003). In Brazil, the family is represented by 228 species distributed in 30 genera, of which 151 are endemic and all of them belong to the neotropical subfamily Gesnerioideae (Flora do Brasil 2020). High endemism and large concentration of taxa are found in the Southeast region of Brazil, mainly in humid forests (Chautems 1988; Chautems and Matsuoka 2003).

Three natural groups or subfamilies were proposed for Gesneriaceae: Coronantheroideae, Gesnerioideae and Didymocarpoideae (Wiehler 1983; Burtt and Wiehler 1995), which are considered monophyletic based on morphological, molecular data (*ndhF* sequences), chromosome number and secondary metabolites (Smith 1996; Smith et al. 1997); however, several studies have shown that groups within Gesneriaceae are not monophyletic (Smith 1996, 2000; Smith et al. 1997; Citerne et al. 2000; Zimmer et al. 2002; Perret et al. 2003; Roalson et al. 2005a, 2005b; Clark et al. 2006, 2012) which resulted in rearrangements in the taxonomic positioning of species, genera and tribes. Three subfamilies are currently accepted for Gesneriaceae: Sanangoideae, Gesnerioideae and Didymocarpoideae (Weber et al. 2013).

The species of Gesnerioideae or Gesnerioid (*sensu* Weber 2004) are neotropical, have equal cotyledonary growth and nectary formed by separate glands (when present). According to Weber et al. (2013) five tribes and their respective subtribes are described: Titanotricheae, Napeantheae, Beslerieae (with Besleriinae and Anethanthinae), Coronanthereae (with Coronantherinae, Mitrariinae and Negriinae), Gesnerieae (with Gesneriinae, Gloxiniinae and Columneinae).

Napeantheae (established by Wiehler 1983) has a single genus, *Napeanthus* Gardner, with about 20 species that are generally found in pits, banks or at the base of rocks in humid and shaded forests at up to 1000 m altitude (Kvist et al. 1998; Lopes et al. 2005; Clark et al. 2020). Its species are herbs with rosulate leaves, without nectaries, and the fruit is a loculicide capsule, characteristics that differentiate them from other Gesnerioideae (Feuillet and Skog 2002; Weber 2004). Napeantheae species have white, pale pink or pale blue actinomorphic flowers (Weber et al. 2020), and have distribution especially in Mexico, Bolivia, Guyanas, Trinidad and Brazil (Moller and Clark 2013; Flora do Brasil

CONTACT Talita Kely Bellonzi talitakb@live.com Faculdade de Filosofia, Ciências e Letras, Programa de Pós-Graduação em Biologia Comparada, Universidade de São Paulo (USP), Ribeirão Preto, SP 14040-901, Brazil.

Published online 17 Mar 2022

2020). The genus Napeathus is represented in Brazil by four native species (Flora do Brasil 2020).

Molecular studies (Smith 2000; Zimmer et al. 2002; Perret et al. 2013; Roalson and Roberts 2016) point that Napeantheae is closely related to Beslerieae, and according to Serrano-Serrano et al. (2017) Napeantheae and Beslerieae are basal lineages within the subfamily. However, Moller and Clark (2013) question the relationship between tribes since Napeantheae can be a basal clade and sister to all other Gesnerioideae (Clark et al. 2010). Thus Moller and Clark (2013) suggested that further molecular and morphological studies will be needed to clarify their taxonomic positioning.

Some studies on Gesneriaceae pollen morphology were carried out, especially for the native Brazilian species: Campos (1962), Melhem and Mauro (1973), Fourny et al. (2010), Gasparino et al. (2011, 2013, 2014), Souza et al. (2018) and Gasparino et al. (2021), including description of pollen from natural hybrids (Araujo et al. 2021). Williams (1978) described a diversity of pollen types for Gesnerioideae in a study with 30 neotropical species, which prompted new studies for the description of pollen grains in the family. According to Smith et al. (1997) the size of the pollen grains supports the monophyly of the Gesnerioideae, however little taxonomic value can be verified for the size of the pollen grains in genera of Gesneriaceae. For Melhem and Mauro (1973), the study of the morphological characters of pollen grains in Gesneriaceae allows for a differentiation of genera, and even species, in some cases, which can be confirmed in subsequent studies (Gasparino et al. 2011, 2013; Souza et al. 2018; Gasparino et al. 2021).

According to Gasparino et al. (2021) the pollen grains in Gesneriaceae are characterized by 3-colpate or 3-colporate apertures and microreticulate to reticulate ornamentation. The majority of Brazilian species have 3-colporate pollen grains, as observed for *Napeanthus primulifolius* (Raddi) Sandwith (Gasparino et al. 2011). Microreticulate ornamentation has been described for most *Sinningia* Nees (Gasparino et al. 2021), in Beslerieae (Gasparino et al. 2011; Cortés-Ceballos et al. 2021), in Napeantheae species (Gasparino et al. 2011) and in *Chautemsia* A.O.Araujo & V.C.Souza, *Goyazia* Taub., *Mandirola* Decne. and *Seemannia* Regel (Souza et al. 2018).

This study aimed to carry out a detailed morphological analysis of pollen grains of Brazilian native species of Napeantheae (Gesneriaceae), since few species were palynologically analyzed and the description of morphological data is necessary for understanding the relationships of tribes and subtribes in Gesneriaceae.

2. Material and methods

We studied the pollen grains of Napeantheae species (*Napeanthus* Gardner) native to Brazil (Table 1). *Napeanthus jelskii* Fritsch and *N. macrostoma* Leeuwenb. occur in the Amazon forest, in the northern region; *N. primulifolius* (Raddi) Sandwith (northeast, south and southeast) and *N. reitzii* (L.B. Sm.) B.L. Burtt ex Leeuwenb. (southern region) are native to the Atlantic Forest of Brazil (Flora do Brasil 2020). The samples of the four species were taken from herborized materials (eight specimes), deposited in the main herbaria of Brazil: INPA, MBM, SP (acronym according to Thiers 2021).

For light microscopy (LM) analysis the pollen grains were acetolyzed according to Erdtman (1960) modified by Melhem et al. (2003) and measured randomly (pollen diameters, $n = 25$, apertures and exine thicknesses, $n = 10$) within the seven-day period (Salgado-Labouriau et al. 1965). For scanning electron microscopy (SEM) pollen grains were used without the acetolysis process, following Melhem et al. (2003). Permanent slides of light microscopy are deposited in the pollen reference collection of the Departamento de Biologia, Universidade Estadual Paulista, UNESP, Jaboticabal, Brazil.

For the metric data of pollen grain diameters, a statistical analysis was performed obtaining the means (x), standard deviation (sx), standard error (s), 95% confidence intervals (CI), coefficient of variability (V) and range of variation (R) according to Vieira (2011) and Zar (2010). For the other measurements (length and width of the apertures and exine) only the arithmetic mean was calculated.

Based on the quantitative data of pollen grains, a multivariate analysis (principal component analysis – PCA) was conducted in order to visualize the grouping of the studied species, for this we used the programs FITOPAC 1 (Shepherd 1996) and PC-ORD version 5.15 (McCune and Mefford 2011);

Table 1. Voucher specimens of Brazilian species of *Napeanthus* Gardner (Napeantheae – Gesneriaceae).

Species	Locality
Napeanthus jelskii Fritsch	GUIANA FRANCESA. Régina, 12 June 1988, C. Feuillet n° 213233 (INPA)
Napeanthus macrostoma Leeuwenb.	BRASIL. Amapá: Rio Jari, 05 August 1961, W.A. Egler & H.S. Irwin n° 272059 (INPA).
	GUIANA FRANCESA. Montagne Bellevie de l'Inini., 21October 1985 (INPA).
Napeanthus primulifolius (Raddi) Sandwith	BRASIL. Iguapé, Reserva ecológica da Juréia, 13 March 1990, M.C.H. Mamede, I. Cordeiro & L. Rossi n° 231 (INPA).
	BRASIL. São Paulo: Caraguatatuba, Parque Estadual da Serra do Mar, 22 October 2000, M.C.H. Mamede, M..R.F. Melo, R.J. Oliveira & F. Marques n° 350545 (SP).
	BRASIL. Bahia: Camacan, 14 October 2014, G.E. Ferreira, A. Chautems, M. Perret & M. Peixoto n° 304 (INPA).
Napeanthus reitzii (L.B. Sm.) B.L. Burtt ex Leeuwenb.	BRASIL. Paraná: Morretes, Morro 7, 17 October 1964 G. Hatschbach s/ n° (MBM).
	BRASIL. Sem local, 23 January 1999, C. Kozera M & V.A. de O. Dittrich n° 910 (INPA).

[INPA = Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brazil; MBM = Museu Botânico Municipal, Curitiba, PR, Brazil; SP = Instituto de Botânica, São Paulo, SP, Brazil].

a cluster analysis (hierarchical cluster analysis – HCA) was also carried out, using Euclidian distances also with software PC-ORD version 5.15 (McCune and Mefford 2011), in order to identify the similarity among the species. For these analyses we used 13 pollen metric variables: length of ectoapertures (ECLE), width of ectoapertures (ECWI), equatorial diameter in equatorial view (EDEV), equatorial diameter in polar view (EDPV), polar diameter in equatorial view (PDEV), length of endoapertures (ENLE), width of endoapertures (ENWI), polar area index (PAI), exine (EXIN), nexine (NEXI), sexine (SEXI), tectum (TECT) and shape (SHAP).

The terminology followed Punt et al. (2007) and Halbritter et al. (2018) and the pollen description was based on what was proposed by Bellonzi et al. (2020). The size and shape classes of pollen grains proposed by Erdtman (1952) were adopted. The definition of the types of amb and their relationship with the apertures followed Erdtman (1952) and Walker and Doyle (1975). The polar area index and the colpi width index followed the one proposed by Faegri and Iversen (1966) and Gasparino et al. (2013), respectively. To standardize the shape of endoapertures, we used Erdtman (1952), Punt et al. (2007) and Soares et al. (2021).

The photomicrographs were performed with a light microscope LEICA DM1000LED, for LM photos, and with a ZEISS EVO MA10 scanning electron microscope for the SEM images.

3. Results

3.1. General pollen description

The pollen grains of the *Napeanthus* (Napeantheae) Brazilian species (Plate 1–2) are monads, isopolar, small, circular to subtriangular amb in polar view, small to very large polar area, suboblate to oblate-spheroidal, tricolporate, circulaperturate, very short, short or long and narrow ectoaperture, rounded at the polar ends, without margo and circular or lolongate endoaperture. The exine is semitectate, microreticulate-fossulate, thin or thick, sexine is thicker than nexine (Tables 2–4).

3.1.1. Size, amb and shape

The species studied presented small pollen grains, the smallest were observed in *Napeanthus primulifolius* and the largest in *N. reitzii* (Table 3); amb (outline in polar view) is circular (*N. reitzii* – Plate 1, Figure 11), subcircular (*N. jelskii* and *N. primulifolius* – Plate 1, Figures 1, 7), or subtriangular (*N. macrostoma* – Plate 1, Figure 4). The pollen grains of *N. primulifolius* have small polar area, *N. jelskii* and *N. reitzii* have pollen grains with large polar area and *N. macrostoma* with very large polar area (Table 4). Suboblate (*N. jelskii* and *N. macrostoma*) and oblate-spheroidal (*N. primulifolius* and *N. reitzii*) pollen grains were observed, however the species presented variations in shape in the same sample (Table 2).

3.1.2. Apertures

All species studied presented 3-colporate pollen grains; long and narrow ectoaperture were observed in *N. primulifolius* pollen grains, *N. jelskii* and *N. reitzii* presented pollen grains with short and narrow ectoapertures and *N. primulifolius* have very short and narrow colpi (Table 4; Plate 1, Figure 9). The ectoapertures are rounded at the polar ends without margo; circular endoapertures were observed, except to *N. macrostoma* pollen grains (with lolongate endoapertures – Table 4).

3.1.3. Exine ornamentation

The pollen grains of *Napeanthus* have semitectate exine with microrreticulate-fossulate ornamentation (Plate 1, Figures 3, 6, 10; Plate 2). In light microscopy it is possible to see the

Table 2. Pollen morphology characterization in Brazilian species of *Napeanthus* Gardner (Napeantheae – Gesneriaceae).

Species	Amb	P/E	Shape (shape variation)	PAI	WCI	Endoaperture
Napeanthus jelskii	Subcircular	0.82	SO* (O, OS)	0.70	9.40	CIR
Napeanthus macrostoma	Subtriangular	0.83	SO* (O, OS)	0.87	16.39	LO
Napeanthus primulifolius	Subcircular	0.98	OS* (SO, SP)	0.45	9.30	CIR
Napeanthus reitzii	Circular	1.00	OS* (SO, PS)	0.62	15.60	CIR

[P/E = ratio between polar and equatorial diameter, SO = suboblate, O = oblate, OS = oblate spheroidal, PS = prolate spheroidal, SP = subprolate, *predominant shape, PAI = polar area index, WCI = width colpus index, CIR = circular, LO = lolongate].

Table 3. Quantitative data of pollen grains diameters of Brazilian species of *Napeanthus* Gardner (Napeantheae – Gesneriaceae, $n = 25$).

Measurements	Species	*Napeanthus jelskii*	*Napeanthus macrostoma*	*Napeanthus primulifolius*	*Napeanthus reitzii*
Polar diameter (VE)	(R) $x \pm s_x$	(14.50–19.00) 16.70 ± 0.22	(12.50–17.50) 14.50 ± 0.29	(12.50–17.50) 13.90 ± 0.29	(17.50–20.00) 18.40 ± 0.24
	s	1.12	1.44	1.46	1.22
	CI	(16.69–16.69)	(13.90–15.10)	(13.30–14.50)	(17.89–18.91)
	V	6.72	9.95	10.49	6.66
Equatorial diameter (VE)	(R) $x \pm s_x$	(17.20–23.50) 20.41 ± 0.34	(15.00–17.50) 17.40 ± 0.10	(12.50–17.50) 14.20 ± 0.28	(15.00–20.00) 18.40 ± 0.28
	s	1.71	0.50	1.39	1.42
	CI	(19.71–20.41)	(17.19–17.61)	(13.63–14.77)	(17.81–18.99)
	V	8.36	2.87	9.80	7.73
Equatorial diameter (VP)	(R) $x \pm s_x$	(16.50–20.50) 18.60 ± 0.25	(15.00–17.50) 17.20 ± 0.17	(12.50–15.00) 14.70 ± 0.17	(17.50–20.00) 19.20 ± 0.24
	s	1.24	0.83	0.83	1.19
	CI	18.12–19.15	16.86–17.54	14.36–15.04	18.71–19.69
	V	6.68	4.82	5.64	6.20

[VE = equatorial view; VP = polar view; R = range; x = mean (μm); s_x = standard deviation (μm); s = standard error (μm); CI = confidential interval in 95% (μm); V = coefficient of variability (%)].

Table 4. Pollen measures of apertures and exine (in μm) of Brazilian species of *Napeanthus* Gardner (Napeantheae – Gesneriaceae, $n = 10$).

	Ectoaperture		Endoaperture					
Species	Length	Width	Length ($x \pm s_x$)	Width ($x \pm s_x$)	Ex	Sex	Nex	Tec
Napeanthus jelskii	2.71	11.80	3.82 ± 0.82	3.59 ± 1.01	1.94	1.35	0.59	0.51
Napeanthus macrostoma	1.06	6.11	3.93 ± 0.64	1.10 ± 0,18	1.26	0.84	0.43	0.25
Napeanthus primulifolius	1.53	10.86	3.94 ± 1.02	3.39 ± 0.61	1.86	1.29	0.57	0.48
Napeanthus reitzii	1.18	15.17	3.81 ± 0.54	2.73 ± 0.62	1.71	1.17	0.55	0.46

[Ex = exine thickness; Sex = sexine thickness; Nex = nexine thickness; Tec = tectum thickness].

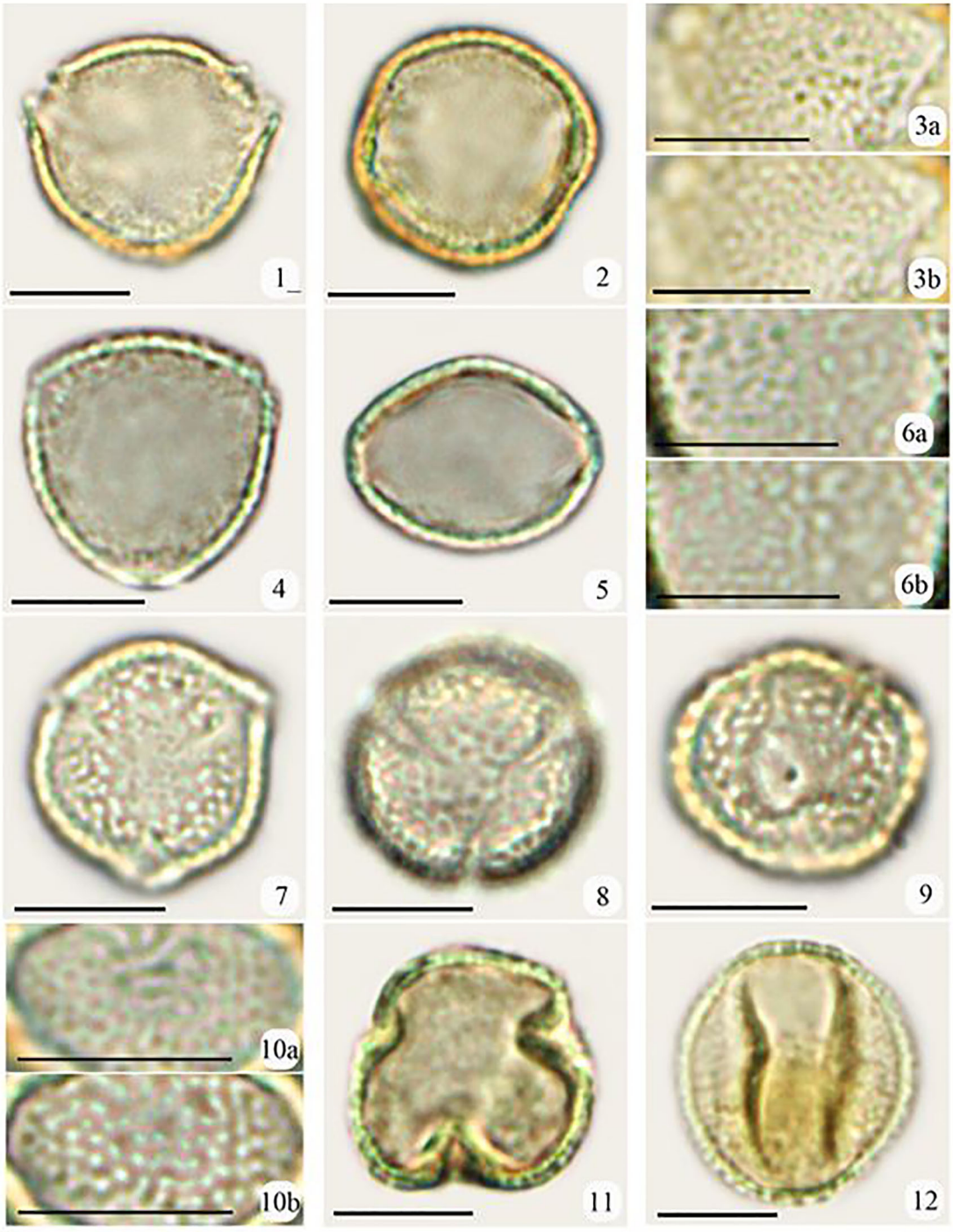

Plate 1. Photomicrographs of the pollen grains of *Napeanthus* Gardner (Napeantheae). Figures 1–3. *Napeanthus jelskii* Fritsch. 1. Polar view, optical section. 2. Equatorial view, optical section. 3a-b. Ornamentation in high and low focus. 4–6. *Napeanthus macrostoma* Leeuwenb. 4. Polar view, optical section. 5. Equatorial view, optical section. 6a-b. Ornamentation in high and low focus. 7–10. *Napeanthus primulifolius* (Raddi) Sandwith. 7. Polar view, optical section. 8. Polar view, apocolpium. 9. Equatorial view, aperture. 10a-b. Ornamentation in high and low focus. 11–12. *Napeanthus reitzii* (L.B. Sm.) B.L. Burtt ex Leeuwenb. 11. Polar view, optical section. 12. Equatorial view, optical section. Scale bars: 10 μm.

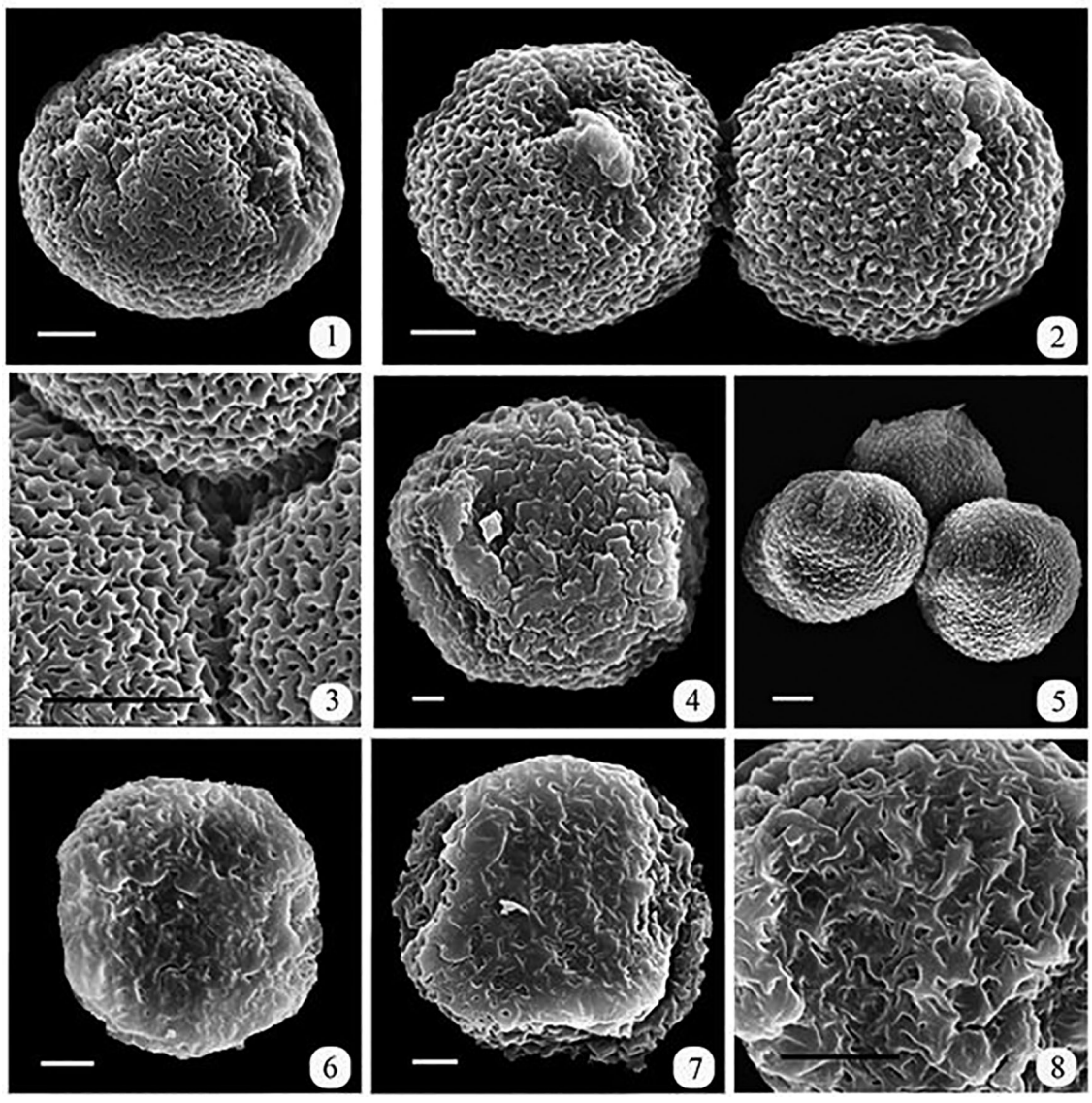

Plate 2. Scanning electromicrographs of the pollen grains of *Napeanthus* Gardner (Napeantheae). Figures 1–3. *Napeanthus jelskii* Fritsch. 1. Polar view. 2. Equatorial view, two pollen grains. 3. Ornamentation details. 4–5. *Napeanthus macrostoma* Leeuwenb. 4. Equatorial view. 5. General view, three pollen grains. 6–8. *Napeanthus primulifolius* (Raddi) Sandwith. 6. Polar view. 7. Equatorial view. 8. Ornamentation details. Scale bars: = 10 μm.

lumina and muri of the microreticulum – LO-analysis (Plate 1, Figures 3, 6, 10), when analyzed in scanning electron microscopy, the pollen grains of *Napeanthus* species show the muri of the microreticulum forming fossulae (Plate 2, Figures 3, 8). Exine is thin or thick (only in *N. primulifolius* pollen grains – Table 4). Sexine is thicker than nexine (Table 4).

3.2. Artificial pollen key to Napeantheae Brazilian species

1. Pollen grains with long ectoapertures … … *Napeanthus primulifolius*
1′. Pollen grains with short or very short ectoapertures … … …. 2
2. Lolongate endoapertures … … … … … … … … …... … … … … … …. … … … … … … … *Napeanthus macrostoma*
2′. Circular endoapertures … … … … … …. 3
3. Amb subcircular and suboblate pollen grains … … … … … … … … … … … … …. *Napeanthus jelskii*
3′. Amb circular and oblate-spheroidal pollen grains … … … … … … … … … … … … … … … *Napeanthus reitzii*

3.3. Multivariate analysis

Thirteen metric variables of pollen from four Brazilian species of *Napeanthus* were used for multivariate analyzed (PCA and cluster analyzed – Figures 1 and 2). The first two axes of the PCA summarized 85.67% of the total variability of the data and the analyzed species were discriminated by their pollen grain measurements. The first axis of the PCA represented 70.91% of the analyzed data (Figure 1), the variables most related to this axis were ENWI, ECWI and ECLE (length of endoaperture and ectoaperture measurements – Table 5), which contribute to the distance of *N. macrostoma* (on the positive side of the axis, with higher values of ENLE and PAI) and the proximity among the other species.

The second axis of the PCA summarized 15.76% of the variability of the analyzed data, where the variables ECLE,

ECWI and PDEV were the ones that most influenced the ordering of the species. Along this axis, the positioning of *Napeanthus jelskii* (on the negative side) influenced by the variable ECLE and of *Napeanthus reitzii* (on the positive side) is highlighted by the influence of shape values (even with low indexes – Table 5).

The Cluster Analysis (Figure 2) elucidated the similarity of the species *Napeanthus primulifolius* and *N. reitzii*, which are similar due to the morphometric data of the pollen grains; these species together show about 80% similarity with *N. jelskii* (Figure 2), which have higher ectoaperture length (ECLE) values when compared to *N. primulifolius* and *N. reitzii*. The *Napeanthus macrostoma* pollen grains in general present different metric data from the other species, especially regarding the endoaperture width values (ENLE – Figure 2).

4. Discussion

Several studies on the pollen morphology of Gesneriaceae have been conducted so far, however, few of them present data on Napeantheae species, especially on Brazilian species.

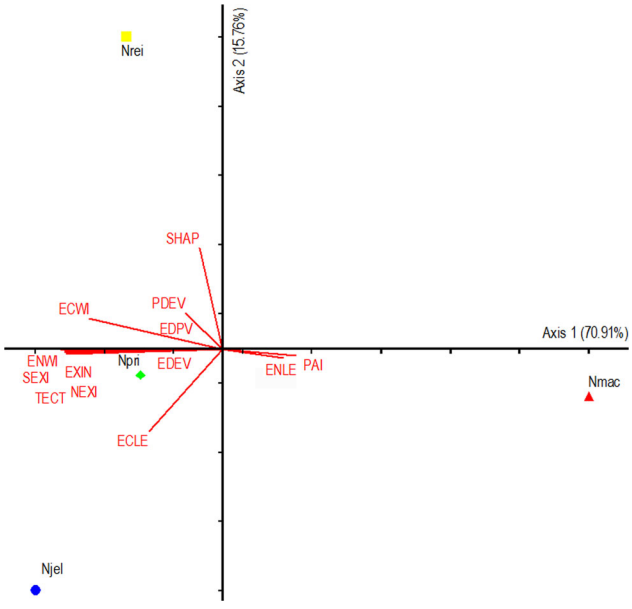

Figure 1. Principal component analysis performed with the pollen metrical variables from Brazilian species of *Napeanthus* Gardner (Napeantheae – Gesneriaceae). ECLE = length of ectoapertures, ECWI = width of ectoapertures, EDEV = equatorial diameter in equatorial view, EDPV = equatorial diameter in polar view, ENLE = length of endoapertures, ENWI = width of endoapertures, EXIN = exine, NEXI = nexine, PDEV = polar diameter in equatorial view, PAI = polar area index, SEXI = sexine. TECT = tectum, SHAP = shape. Njel = *Napeanthus jelskii*, Nmac = *Napeanthus macrostoma*, Npri = *Napeanthus primulifolius*, Nrei = *Napeanthus reitzii*.

Thus, the present study presents the pollen morphology data of Napeantheae taxa native to Brazil, contributing to the palynology of the family in one of its diversity centers.

Williams (1978) described for *Napeanthus costaricensis* isopolar and reticulate pollen grains like those observed by him in Beslerieae and Gloxinieae, however the author cited that for Napeantheae no differences in ornamentation between mesocolpium and apocolpium were observed. When comparing the results of Williams (1978) and those observed here, a similarity is noted in terms of ornamentation, and it is possible to see in the images of the study by Williams (1978) ripples in the muri of the microreticulum of the pollen grains of *N. costaricensis*, which can characterize microreticulate-fossulate ornamentation as described in the present study. It is important to mention that the distinction between reticulum and microreticulum was proposed by Praglowski and Punt (1973) but only widely used after the glossaries by Punt et al. (1994, 2007), so for the study by Williams (1978) these definitions were often used synonymously.

Gasparino et al. (2011) defined to *Napeanthus primulifolius* and *N. reitzii* pollen grains a microreticulate ornamentation, with sinuous muri in the microreticulum. According to Gasparino et al. (2021) the microreticulate exine is characteristic for several genera in Gesneriaceae (as well as reticulate ornamentation), however the muri sinuous of the microreticulum observed here delimit an elongated and irregular groove, which configures a fossulate ornamentation formed by the microreticulum. Thus, the microreticulate-fossulate ornamentation defined here seems to be unique to *Napeanthus* pollen grains when compared to other genera of Gesneriaceae (E.C.Gasparino, T.K.Bellonzi and C.N.Souza, personal communication).

Details of the apertures of the pollen grains of Napeantheae were not explored in the study by Williams (1978). The results of our study on pollen apertures corroborate the data described by Gasparino et al. (2011) with 3-colporate pollen grains for *Napeanthus* species. We observed that the length of pollen ectoapertures may vary in *Napeanthus* (long in *N. primulifolius* and short or very short in other species); this result confirms Weber's (2004) statement that indicates variation in the length of ectoapertures for New World Gesneriaceae species.

Lolongate endoapertures as observed here only for the pollen grains of *N. macrostoma* were also described for *N. primulifolius* and *N. reitzii* in the study by Gasparino et al. (2011), indicating a divergence in the data. If we compare only the mean of the endoaperture measurements, the pollen grains of *N. primulifolius* and *N. reitzii* analyzed here would present lolongate endoapertures, however, this study

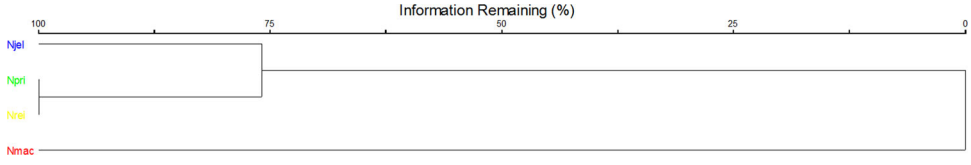

Figure 2. Cluster analysis (UPGMA and Euclidean distance) performed with the pollen metrical variables from Brazilian species of *Napeanthus* Gardner (Napeantheae – Gesneriaceae). Njel = *Napeanthus jelskii*, Nmac = *Napeanthus macrostoma*, Npri = *Napeanthus primulifolius*, Nrei = *Napeanthus reitzii*.

Table 5. Pearson correlation coefficient for pollen grains metric variables of the first and the second axis of PCA ordination of *Napeanthus* Gardner (Napeantheae – Gesneriaceae) Brazilian species.

		Principal components	
Variables		Axis 1	Axis 2
ECLE	Length of ectoapertures	−0.327	−0.730
ECWI	Width of ectoapertures	−0.570	0.578
EDEV	Equatorial diameter in equatorial view	−0.043	−0.085
EDPV	Equatorial diameter in polar view	−0.029	0.104
ENLE	Length of endoapertures	0.016	−0.013
ENWI	Width of endoapertures	−0.647	−0.154
EXIN	Exine	−0.212	−0.071
NEXI	Nexine	−0.087	−0.015
PDEV	Polar diameter in equatorial view	−0.106	0.221
PAI	Polar area index	0.130	−0.088
SEXI	Sexine	−0.196	−0.072
TECT	Tectum	−0.158	−0.007
SHAP	Shape	−0.034	0.153

chose to follow the proposal by Soares et al. (2021) considering the use of statistics for the definition, allowing the presentation of more accurate data. Our data also corroborate those of Gasparino et al. (2011) regarding the oblate-spheroidal form of pollen grains from *N. primulifolius* and *N. reitzii*.

According to Wiehler (1983) *Napeanthus* species have already been treated in other tribes and other genera of Gesneriaceae, such as *Phinaea* Bentham and *Niphaeae* Lindley– Gloxiniinae, and *Episcia* Martius– Columneinae (Ivanina 1965), and also in other botanical families, such as Gentianaceae, Scrophulariaceae and Oxalidaceae (Karsten 1860; Smith 1953; Sandwith 1956); however, palynology allows us to distinguish *Napeanthus* pollen grains from these genera and families. There are differences in ornamentation, types and aperture details when comparing pollen grains from *Napeanthus* with those from other families (Santos and Melhem 1998, 1999 – Scrophulariaceae; Rosenfeldt and Galati 2007; Correa and Fonseca 2015 – Oxalidaceae). Furthermore, variations in the aperture details and especially in the type of ornamentation of *Napeanthus* pollen grains, as mentioned above, were not observed among the genera of Gesneriaceae (Williams 1978; Gasparino et al. 2011, 2013; Souza et al. 2018; Gasparino et al. 2021).

5. Conclusion

Our study indicates that variations in shape, amb, length of ectoapertures and type of endoapertures were the morphological characteristics of the pollen grains that allowed the distinction of the Brazilian species of *Napeanthus* (Napeantheae) analyzed. The multivariate analysis confirmed the importance of measuring the diameters and apertures of the pollen grains analyzed, which helps in the palynology of the genus. The microreticulate-fossulate ornamentation is the diagnostic character of *Napeanthus* pollen among the other Gesneriaceae.

Acknowledgements

The authors are grateful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting scholarship to the first author, and the Herbaria curators for kindly allowing pollen sampling for the present study. The authors are thank the Laboratório de Microscopia Eletrônica, FCAV for providing technical assistance with scanning electron microscopy.

Disclosure statement

No potential conflicts of interest were reported by the authors.

ORCID

Talita Kely Bellonzi http://orcid.org/0000-0003-2586-4238
Eduardo Custódio Gasparino http://orcid.org/0000-0001-6078-7341

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